“Biogenic Synthesis of Nanoparticles and Evaluation for Antimicrobial Activities”

A Synopsis

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Introduction

The nanoparticles were rod in shape and size of 25-80nm with an average size of 52.4nm. Different types of nonmaterial like Zinc, Silver, Copper, Alginate Gold, and Platinum. Silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial activity against Bacteria, Virus and Eukaryotic micro-organism (Zahir *et al.,* 2012). Thus it is the need to search or develop a suitable Biogenic Green Synthesis of Nanoparticles and In-vitro Evaluation of their Antimicrobial Activity. Developing the biological synthesis and environmental benign technology for the green synthesis of nanoparticles. Nanoparticles are being considered as fundamental building blocks in nanotechnology. The use of nanoparticles is gaining impetus in the present century they can posse's defined optical, chemical & mechanical properties. The nanoparticles are most promising as they contain remarked antimicrobial activity due to their large surface area to volume ratio, which is the interest to researcher due to the growing microbial resistant against antibiotics and development of resistant strain (Praveen *et al.,* 2012). Some application of nanomaterial to biology, medicine, agriculture, is fluorescent biological labels drug and gene delivery antimicrobial and anti-insect molecules. Bio-nanotechnology combine with biological, physical & chemical approaches to produced nanosized particles with specific function. It also represent in economic substitute for chemical and physical method of nanoparticles formation (Piao *et al.,* 2011). Nanoparticles exhibit completely new properties based on specific characters such as size distribution and morphology. Specific surface is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. This method of synthesis can be divided in to intracellular and extracellular with following steps. Which must be evaluated based a green synthesis of chemistry perspective including (a) selection of solvent medium (b) selection of environmentally benign reducing agent (c) selection of nano-toxic substances for the AgNPs stability (Krishnamurthy *et al.,* 2012). Among the various biogenic approaches, the use of plant extract has advantages such as easily available safe to handle and passes a broad viability of metabolites. It has been reported that medicinally valuable angiosperms have the greatest potential for the synthesis of nanoparticles with to the respect of quantity and quality (Singh *et al.,* 2012). The common approach for green synthesis at ambient temperature is to begin with naturally available resources containing phyto-chemically that function as both the reducing and stabilizing agent (Savithramma *et al.,* 2011).

Defined production condition is crucial to obtain adequate size of nanoparticles. Particles size, chemical composition, crystalline and shape can be controlled by temperature, pH value, concentration, chemical composition surface modification and process control two basic are uses to produced nano particles "Top down" and "Bottom-up"(Singh and Bhakat 2012). Production of nanoparticles generally falls in to four categories: i} solid phase particles ii} size reduction iii} liquid phase synthesis iv} gas phase synthesis. In addition to economic consideration each process suffer from limitation ranging from poor property contraltos the introduction of outside contaminants in to the products (Johan *et al.,* 2012).

In the present study "green" pathway will be used to produce nanoparticles by using the regionally significantly bioresources. Green synthesis of nanoparticles will be extensively

carried out by using plant extract which have properties of emulsifiers. In green synthesis we will use natural reducing agent synthesized nanoparticles (Prakash and Thiagarajan 2012). Most of the reported green synthesis method using plant took more than 1-hour for the formation of colloidal silver green synthesis of nanoparticles and their characterization is an emerging field of nanotechnology from the post few decades because of their application in the field of physics, chemistry biology, and medicine. The development of green processes for the synthesis of nanoparticles is evolving in to an important branch of nanotechnology (Sharma *et al.,* 2008).

Pathway to the green synthesis produced nanoparticles using by following methods.

(1) Polysaccharide method

(2) Tollens method

(3) Irradiation method

(4) Biological method

Biological method of synthesis have way for the "greener synthesis" of nanoparticles and these have proven to be better method due to slower kinetics they after better manipulation and control over crystal growth and stabilization. This has motivated an up surge in research on the synthesis routes that allow better control of shape and size for various nanotechnology applications. The use of environmentally benign material like plant extract, fungi, bacteria and enzyme for the synthesis of silver nanoparticles and compatibility of pharmaceutical and other biomedical application as they do not use toxic chemical for the synthesis protocol (Prashanth *et al.*, 2012). A variety of techniques including physical and chemical method have been developed to synthesize of nanoparticles. Physical method is highly expensive and chemical method is harmful to the environment (Malabadi *et al.*, 2012).

The main photochemical synthesis for the nanoparticles is terpenoids flavones, aldehyde, and amides etc. Every plant contains thousand of various alkaloids, steroids, flavonoids terpenoids and other molecules with biological activity. The synthesis of nanoparticles will be evaluated for antimicrobial activity and further characterize for these size and shape by (SEM, TEM, FTIR, XRD, UV-Vis spectra) (Mary and Inbathamizh 2010). The conventional methods for synthesizing nanoparticles are usually hazardous. Which stimulate the need for synthesizing the natural resourse. This lead to focus on "green synthesis" og nanoparticles which seem to be easy efficient and eco-friendly approaches. The study of green synthesis of nanoparticles will be carried out using the plant extract of a using agent (Singhal 2012). The nanoparticles synthesis will be characterized using UV-Vis Spectroscopy, Scanning Electron Microscopy, and Transmission Electron Microscopy etc analysis. During the past two decades, the interaction of a variety of plant, bacteria, fungi, actinomycetes and algae in the synthesis of silver nanoparticles have been well investigated. Plants, fungi and bacteria have huge potential for the production of silver nanoparticles; however, the previous studies are still largely in the discovery phase. Given the anticipated wide application of silver nanoparticles for commercial applications, in the field of agriculture application of bacteria, cyanobacteria, algae, plant that minimizes hazard and waste will be essential for the transition of nanoscience discoveries to commercial products of nanotechnology (Pawlyta *et al*,2012). Future work should implement systematic experiments which include development of silver

nanoparticles of well-defined size and shape. Better understanding of the mechanisms of silver biosynthesis will enable us to achieve better control over size, shape, and mono-disparity which will lead to the development of high precision in the production level and the application of nanoparticles for commercial scale agricultural applications in the management of plant disease (Mishra and Mishra 2011).

Importance of Nanoparticles

1- It is used in paints, cosmetics, sunscreens, plastic and rubber manufacturing electronics and pharmaceuticals products etc.

2- It is also potentially used to treat leukemia and carcinoma cancer cell

3- It is also a strong antimicrobial agent

4- It is also used as drug carrier

5- It is used for purification and quality management of air, biosensing imaging, drug delivery system.

Antimicrobial Activity of Nanoparticles

Antimicrobial agents are broadly of two types organic and inorganic at high temperature pressures organic antimicroal materials are formed to be less stable compared to inorganic antimicrobial agent. Current research in inorganic nanomaterials having good antimicrobial properties has opened a new area in pharmaceutical and medical industries.

Application of Nanoparticles

1- Drug delivery

2- As medical diagnostic tools

3- As a cancer treatment agent

4- Human health care

5- Catalytic action

Review of Literature

The term of nanotechnology was coined by Professor Noerio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacture of material at the nanometer level. The concept of nanotechnology was given by physicist Professor Richard P. Feynman in his lecture there's plenty of room at the bottom (Dhanlakshmi and Rajendran 2012). Nanoparticles are ranging in the size of 10-200nm, and are in the solid state either amorphous or crystalline in nature. Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity of silver nanoparticles (Sivakumar *et al*., 2011). As specific area of nanoparticles is increased their biological effectiveness can increases due to the increases in surface energy. They are able to absorb or encapsulated a drug or a chemical protecting against chemical and enzymatic degradation (Singh *et al.,* 2008).

In recently, biodegradable polymeric nanoparticles have many applications in modern medicine in term of controlled drug delivery of protein, peptides through a designated route. In general nanoparticles were synthesized from different synthetic polymers polylactide(PLA), polyglycolide(PLG), & poly(D,L-lactide-co-glycoside)(PLGA), nanoparticles represent the most extensively investigated ones.Further some of promising polymers approaches are poly-cyanoacrylate(PCA), poly-alkylcyanoacrylate(PACA), poly ε-caprolactone and poly ester-anhydride (Krishnamurthy *et al.,* 2012). In addition to these polymers, natural biopolymers and macromolecules such as chitosan and gelatin were also used for the synthesis or nanoparticles. They synthesis of nanoparticles and their self-assembly is a cornerstone of nanotechnology (Singh *et al.,* 2011). Type of nanoparticles Silver, Gold, Zinc, Titanium, Magnesium Copper, & platinum etc. Nanoparticles have proved to be most effective as it has good antimicrobial efficiency against bacteria and other microorganism (Savithramma *et al.,* 2011).

Medicinal use of Nanoparticles: The size of nano-material is similar to that of most biological, molecules and structure; therefore, nano-material can be used for both in-vivo and in-vitro biomedical research and application (Dhas *et al.,* 1998). The use of nanoparticles in medicine and more specifically drug delivery is set to spread rapidly. Currently many substances are under investigation for drug delivery and more specifically for cancer therapy (Johan *et al.,* 2012).One of the many field that nanotechnology is making the field of medicine and that method are used sensing and diagnostics within the body method of injecting drugs into the body without any risk of infection. Nanoparticles made for the good diagnostic tool because they can reach lots of places within the body easily through the blood stream and also very sensitive due to the size (Prakash and Thiagarajan 2012).

Magnesium Nanoparticles: Mg nanoparticles have received intense attention due to their novel properties different from bulk material and various applications in fields of H_2 storage battery, composite filler's etc. Magnesium nanoparticles are prepared by two main methods high energy ball milling and the gas-condensation methods (Sharma *et al.,* 2008). Magnesium

is a light abundant and a relatively cheap metal which is attractive for hydrogen storages due to the reversible gravimetrical capacity of 7.6wt% hydrogen. The preparation of magnesium nanoparticles with size below 5nm by various routes, Example: Electrochemical reduction solvent metal atom dispersion (SMAD) carbon supported melt infiltration, and a sono-electrochemical method (Prashanth *et al.,* 2011). It has been reported that the desorption temperature Td can be decreased to 85°C and 115°C compared to Td>350°C for bulk magnesium. The size of magnesium crystallites is directly influenced by the pore size of the carbon and can be varied from 2-5 to less than 2nm. The majority of the nano-crystallites are not oxidized after preparation (No bulk magnesium was detected in the sample with nanoparticles leading up to 15wt % on carbon) (Malabadi *et al.,* 2012).

Copper Nanoparticles: Copper nanoparticles are prepared by sol-gel method using Hydrazine Hydrate (HH) and Sodium Formaldehyde Sulfoxylate (SFS) in order to prepare nano copper. That copper has faced centered cubic structure and their size is ~6nm (Mary and Inbathamizh 2010). Therefore according to the Hume-Rothery rule copper should from solid solution. Nanoparticles of expensive based metals, such as copper nanoparticles have recently attracted much attention as an innovative nano material for application such as highly-active gas reforming catalysts for hydrogen production (Singhal 2012).

Gold Nanoparticles: The preparation of nano-scaled gold material has become very important due to their unique properties, which are different from those of the bulk material. The properties of these particles in application as diverse as catalysis, sensors and medicine depend critically on the size and composition of nanoparticles production of nanoparticles can be achieved through mainly three method such as chemical, physical and biological method(Pawlyta *et al.,*2012). Since noble metal nanoparticles such as Gold, Silver, & Platinum etc nanoparticles. There is a growing need to developed environmentally friendly processes of nanoparticles synthesis that do not used toxic chemical (Mishra and Mishra 2011). Nanoparticles it can be divided by two group of method organic and inorganic nanoparticles, due to their size of features and advantages ever available chemical imaging drug agent and drugs inorganic nanoparticles have been examined as potential tools for medicinal imaging as well as for treating diseases inorganic material have been widely used for cellular delivery due to their versatile features of wide availability, good biocompatibility, capability of targeted drug delivery and controlled release of drugs. Gold nanoparticles have been used extensively in imaging as drug carriers and in thermo-therapy of biological targets (Dhanalakshmi and Rajendran 2012).

As a specific surface is relevant for catalytic reactivity and other related properties such as antimicrobial activity in gold nanoparticles. As specific surface area of nanoparticles is increased in surface energy. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo-therapy of biological targets (Sivakumar *et al.,* 2011). Nanoparticles based on gold chemistry are an immense area of research is widely used in various field of application. They are versatile agents with numeras application in biomedicine like diagnostics assay thermal ablation, radiotherapy enhancement, gene and drug delivery etc. Gold nanoparticles have been used for the detection of antigen in conjugation with an antibody (Singh *et al.,* 2008).The conjugation of gold nanoparticles with

biological important molecules like Oligosaccharide, DNA, and Protein has made a great deal of impetus in recent days (Michael whitehead 2012).

Silver Nanoparticles: That silver nanoparticles exhibits potent antibacterial properties with low toxicity for human and Animals by comparison with other heavy metals. Ag and Ag-compound material are effective for gram negative and positive bacteria, whereas the efficiency of conventional antibiotics varies with the species of bacteria, many worker have recently reported that Silver nanoparticles demonstrate excellent antibacterial activity (Krishnan *et al.,* 2010). Silver nanoparticles show efficient antimicrobial compared to other salt due to their extremely large surface area, which provides better contact with microorganism. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria (De jongh *et al.,* 2007). The bacterial membrane and also penetrates inside the bacteria the bacterial membrane contain sulfur containing protein and silver nanoparticles interact with these protein in the cell as well as with the phosphorus containing compound like DNA (Dhas *et al.,*1998). When silver nanoparticles enter the bacterial cell. It form a low molecular weight region in the center of bacteria to which the bacteria conglomerates thus, protective the DNA from the respiratory chain, cell division finally leading to the cell death (Nagaraj et al., 2012). The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity. Nanoparticles applications are increasing rapidly nano crystalline silver particles have found tremendous application in high sensitivity bimolecular detection and diagnostic antimicrobial and electronics (Grace and Tandian 2007). There is still need for economics commercially doable in addition environment friendly synthesis route to synthesize silver nanoparticles there for the synthesis of silver nanoparticles example: chemical reduction photochemical reverse micelles, Thermal decomposition and green chemistry method (Udayasoorian *et al.,* 2011).

Platinum nanoparticles: Platinum is an important row material with many application examples: catalyst for co-oxidation in catalytic converters and for fuel cell technology (Sivakumar *et al.,* 2012). One of the method is to produce platinum nanocrystal's size of nanocrystals is connected with specific surface area decreasing of the average in increase of value specific surface area (Meruva *et al*., 2011). A second factor which motivates nano material investigation, including platinum is that some properties of material in nano-scale strongly depend on size and is smaller than 100nm. Because of nano-matrix sizes image such nanostructure (Yoo whan *et al.,* 2004). That platinum prepared by citrate reduction has the ability to quench superoxide anion radical and hydrogen peroxide. Although the quenching mechanisms of cation have not been elucidated some data indicate that they may be quenched by a catalytic redox- reaction coupled with an electron transport. Electron transfer by platinum nanoparticles has been demonstrated when they catalyzed the reaction between hexacynoferrates and thiosulfate ions. Moreover firstly some studies have that Gold nanoparticles catalyzed the oxidation of NADH to NAD^+ (Cushine and lamb 2005).

Zinc Nanoparticles: Zinc oxide Nanoparticles is an interesting semiconductor material due to its application solar cells, gas sensor, ceramics catalysis, & cosmetics etc. In this work the precipitation method was used by controlled and freezing drying processer. The material obtained were thermally treated a various temperature (Ahmad *et al.,* 2010). The influence of

temperature on structural, textural, and morphological properties of the material was studies by powder X-ray diffraction, Infrared spectroscopy, Scanning Electron Microscopy, Adsorption and Thermal Analysis, certain chemical can interfere directly with the proliferation of microorganism at concentration that can be tolerated by the host (Sastry *et al.,* 2003).

Zinc deficiency is a major problem for both plant and human health understanding the physiological effect and function of zinc in plant system is needed to find effective ways to increase the available levels of zinc in soils to improve crop productivity and to increases the bio-available level of zinc in edible portion of food crop of that the feed the world's resource poor people (Vahabi *et al.,* 2012). The role of zinc stabilizing cell membrane is still not completely clean but appears to be related to zinc ability to protect membrane protein sulfohydryl group in cystine residue from oxidation by other transition metal to the disulfide amino acid, cystine more research is needed to understand this important function of zinc in plant cell membrane (Alappat *et al.,* 2012).

Antimicrobial Agent: The antimicrobial agent has become increasing important and pressing global problem of the two million people who acquire bacterial infection in US hospital each year 70% of cases now involve strain that are resistant to at least one drug. Structural modification of antimicrobial drugs to which resistances developed has proven to be an effective means of extending the life span of antifungal agent such as the azoles antiviral agent such as the nono-nucleoside reverse transcriptase inhibitors, and various antibacterial agent including β-lactoms and quinolones (Moharrer *et al.,*2012). Those in responses to antimicrobial resistance major pharmaceutical companies have tended to concentrate their effort on improving antimicrobial agent. However, with the portfolio of chemotherapeutics currently available those researchers are close to the end of the term parent structure alterations nanotechnology (Sangappa *et al.,* 2012).

Objectives

1. Collection of Plant materials and their Identification.

2. Isolation and identification of bioactive compounds by different qualitative methods.

3. Green synthesis of plant-mediated nanoparticles using e.g., *Punica granatum Eleocarpus ganitrus, Terminalia arjuna, Commiphora mukul* and *Emblica officinalisa.*

4. Charaterization Studies of synthesised Nanoparticles.

5. To Evaluate the Antimicrobial activity of synthesised Nanoparticles against selected microorganisms

6. Comparison of Plant mediated Nanoparticles with standard drugs.

Methodology:

1. *Punica granatum* fruit peels will be collected from local market of Ludhiana Punjab and *Eleocarpus ganitrus* Seeds, *Emblica officinalis* leaves, *Terminalia arjuna* leaves, *Commiphora mukul* leaves plant will be collected from Jayoti Vidyapeeth Women's University, Jaipur. Identification of plants will be carried out with the help of Department of Botany, University of Rajasthan, Jaipur.

2. *Punica granatum* fruit peels, *Eleocarpus ganitrus* seeds, *Terminalia arjuna* leaves, *Commiphora mukul* leaves and *Emblica officinalis* leaves will be dried. The fine powder will be used as herbal drug. Weighed amount of dried powdered of whole plant will be extracted to exhaustion in a Soxhlet apparatus.

3. Qualitative study will be extracted from crude and aqueous extract of plant material by following the method of (Ojieh *et al*., 2013).

4. Biosynthesis of four types of nanoparticles (Silver, Copper, Zinc, Iron) by following the method of Praveen *et al*., 2012.

5. Screening of synthesized nanoparticles against tested microorganism will be done by Disc Diffusion Method.

6. Characterization of synthesized nanoparticles by advanced techniques i.e. TEM, SEM, FTIR, and XRD.

7. Data will be tabulated and analyzed statistically.

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